

Detection of Extraterrestrial Life  
Method II: Optical Rotatory Dispersion

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## 1.0 INTRODUCTION

The object of this study is to develop polarimetric methods to detect the presence of DNA (deoxyribonucleic acid) or its congeners in soil suspensions, and through these methods determine the existence of life (as known terrestrially) on other planets. The cotton region associated with optically active organic compounds is being used to detect and characterize the compounds above.

In the previous report to NASA (First Quarterly Report, 20 December 1962 to 19 March 1963), it was shown that there are certain components within the water soluble extract of soil that exhibit anomalous dispersion. Also, the theoretical development of a new electronic ratio, the difference to a constant, for measuring optical activity was presented. It was shown in this development that optical activity measurements may be made in the presence of circular dichroism, which is defined as the unequal absorption of right and left circularly polarized light, absorption of light due to a chromophore, and scattering.

The work at present is concerned both with the instrumentation necessary to measure this new electronic ratio and the extraction of the optically active components from the soil.

The Rouy-type polarimeter is being used at present to obtain optical rotatory dispersion data on these extracts. Certain problems have materialized which indicate this polarimeter to be a qualitative instrument. These problem areas are as follows:

(a) The presence of reflecting surfaces which are located between the polarizer prisms and analyzer prisms result in the generation

of elliptically polarized light. The amount of elliptically polarized light generated depends upon the angle which the plane polarized beam makes with the reflecting surfaces. This elliptically polarized light can pass through the analyzer prism. Since the Rouy-type polarimeter contains two polarizing prisms, the positioning of the optic axis of these prisms with respect to the reflecting surfaces is important. If the optic axis of the polarizing prisms are at different angles with respect to the reflecting surfaces, different intensities of light will pass through the analyzer prism as a result of the generation of elliptically polarized light.

(b) The state of the art in cutting the glan prisms has not achieved the state necessary for making optical rotatory measurements. The transmission of these prisms decreases as a function of wave length. Also the transmission of two glass prisms at designated wave lengths cannot be matched.

These problems apparently will not enter into the measurement of rotatory dispersion by means of the new electronic ratio, the difference to a constant. The reasons for this statement will be discussed later in this report.

## 2.0 BACKGROUND

It is generally known that the organic matter in soil may be found in the fulvic acid or humic acid fractions. The fulvic acid fraction is considered as acid soluble whereas the humic acid fraction is considered as base soluble. Recently, Anderson (1,2) was able to detect and estimate the amount of purines and pyrimidines of DNA in the humic acid fraction. This was done by heating this fraction with concentrated perchloric acid.

Under these conditions, DNA is readily degraded to these derivatives. It was also pointed out that the RNA which may be present in the soil may be found in the fulvic acid fraction. The reason for this is fairly obvious. The RNA readily undergoes hydrolysis to form the mononucleotides in basic media. Solubilization of the humic acid fraction in basic media and reprecipitation of this fraction in acid media permits its separation from the fulvic acid fraction. Since the mononucleotides are acid soluble, they remain in the fulvic acid fraction.

It was mentioned earlier that a new electronic ratio, the difference to a constant, which is proportional to optical rotation by means of a simple functional relationship has been developed. This ratio can readily be measured on a double beam instrument in which one beam contains both a polarizer and an analyzer prism; the other beam contains only a polarizer prism. By setting the optical axis of the analyzer at an angle of  $(+\theta)$  and then at  $(-\theta)$  with respect to the optical axis of the polarizer, optical rotation in the presence of absorption, scattering and circular dichroism may be determined. The key to this method is to direct one beam of plane polarized light through a sample and allow this beam to fall on an analyzer prism. The light which passes through the analyzer then impinges on a photo multiplier tube. The other beam which contains only the polarizing prism and sample also impinges on a photo multiplier tube. The ratio of the currents which are produced by the photomultiplier tubes give a function which is linear with respect to the optical activity of the sample. The relationship which expresses the ratio of, the difference to a constant, in terms of optical activity is represented by equation (1).

$$(1) \quad R = 4t\alpha \left[ 1 - \frac{(\Delta K l c)^2}{8} \right]$$

where

R = the ratio of the difference to a constant

$\alpha$  = angular rotation of the sample in radians

t =  $\tan \theta$ , where  $\theta$  is the angle between the optical axis of the analyzer and the optical axis of the polarizer

$\Delta K$  = difference in absorption coefficients associated with circular dichroism, the unequal absorption of right and left circularly polarized light by an optically active sample.

l = path length

c = concentration of the optically active solution

From equation (1), it can readily be shown that for a sample with an angular rotation of about 0.1 radian and  $\theta = 45^\circ$ , the departure from linearity of the ratio of the, difference to a constant, in terms of angular rotation is less than 0.7%.

(1) Anderson, G., Soil Science, 86, 169 (1958)

(2) Anderson, G., Ibid, 89, 156 (1961)

### 3.0 EXPERIMENTAL PROCEDURES

All rotatory-dispersion measurements were performed on the Rouy-type polarimeter. To eliminate part of the stray white light from the monochromator, a corning filter, No. 9868, was used.

A series of soil samples obtained in the vicinity of Melpar were investigated. The initial acid extraction was prepared by adding



0.15 N hydrochloric acid to these soils. The concentration of these mixtures were 0.1 g of soil per ml of acid. The base soluble extracts were prepared by adding 1 ml of 0.15 N sodium hydroxide to every 0.01 grams of soil. The fulvic acid fractions were extracted from the soils by initial treatment with 0.15 N sodium hydroxide, separation of the base soluble fraction from the insoluble components, and reprecipitating the humic acid fraction with 0.15 N hydrochloric acid. The concentration of the remaining acid soluble fulvic acid fraction was based on 0.02 grams of soil per 1 ml of final acid solution. The pH of the initial acid extracts, the base soluble extracts and the fulvic acid extracts were 2.8, 8.4 and 4.3, respectively.

All mixtures were stirred for 12 hours on a magnetic stirrer. Initial separation of the soluble fraction from the insoluble components was done with an International Portable Refrigerated centrifuge Model PR-2. After one hour, the supernatant was filtered through a Millipore filter, pore size 0.45 microns.

The hydrolysis reaction of the humic acid fraction with concentrated perchloric acid was accomplished in the following manner. One gram of the humic acid fraction obtained by the method discussed previously was dissolved in 50 ml of 0.15 N sodium hydroxide. This fraction was then reprecipitated with 0.15 N hydrochloric acid and dried overnight. Twenty ml of 72% by weight of reagent grade perchloric acid was added to 20 mg of the dried humic acid fraction. This mixture was heated for one hour in a boiling water bath. The solution was then allowed to cool.

The optical rotatory dispersion measurements were performed on this solution without further dilution.

#### 4.0 RESULTS

All optical rotatory dispersion measurements on the extracts of soil were performed on the Rouy-type polarimeter. It must be kept in mind that these measurements are qualitative for the reasons explained earlier in this report. However, valuable information is indicated from the optical dispersion measurements obtained on the acid and base extracts of soil.

It was pointed out earlier in this report that the organic matter in soil may be extracted by acidic and alkaline treatment of soil. The dispersion curves in Figure 1 represent the measurements performed on the initial acid extract (curve 1), the basic or humic acid extract (curve 2) and finally the acid soluble components or fulvic acid fraction (curve 3) remaining in solution after the humic acid fraction was reprecipitated with an acid solution. It is of interest to compare the R maximum obtained from humic acid extract with the R maximum obtained from the initial acid extract. These are 1.14 and 7.4, respectively. The R-maximum may be considered as being directly related to optical rotation in a qualitative manner. Although, the R maximum for the humic acid extract is approximately twice that of the initial acid extract, the amount of soil necessary to produce the humic acid dispersion curve is one tenth of that required to produce the dispersion curve for the initial acid fraction. In addition, the R maximum for the dispersion curve of the fulvic acid fraction is approximately 0.7. To

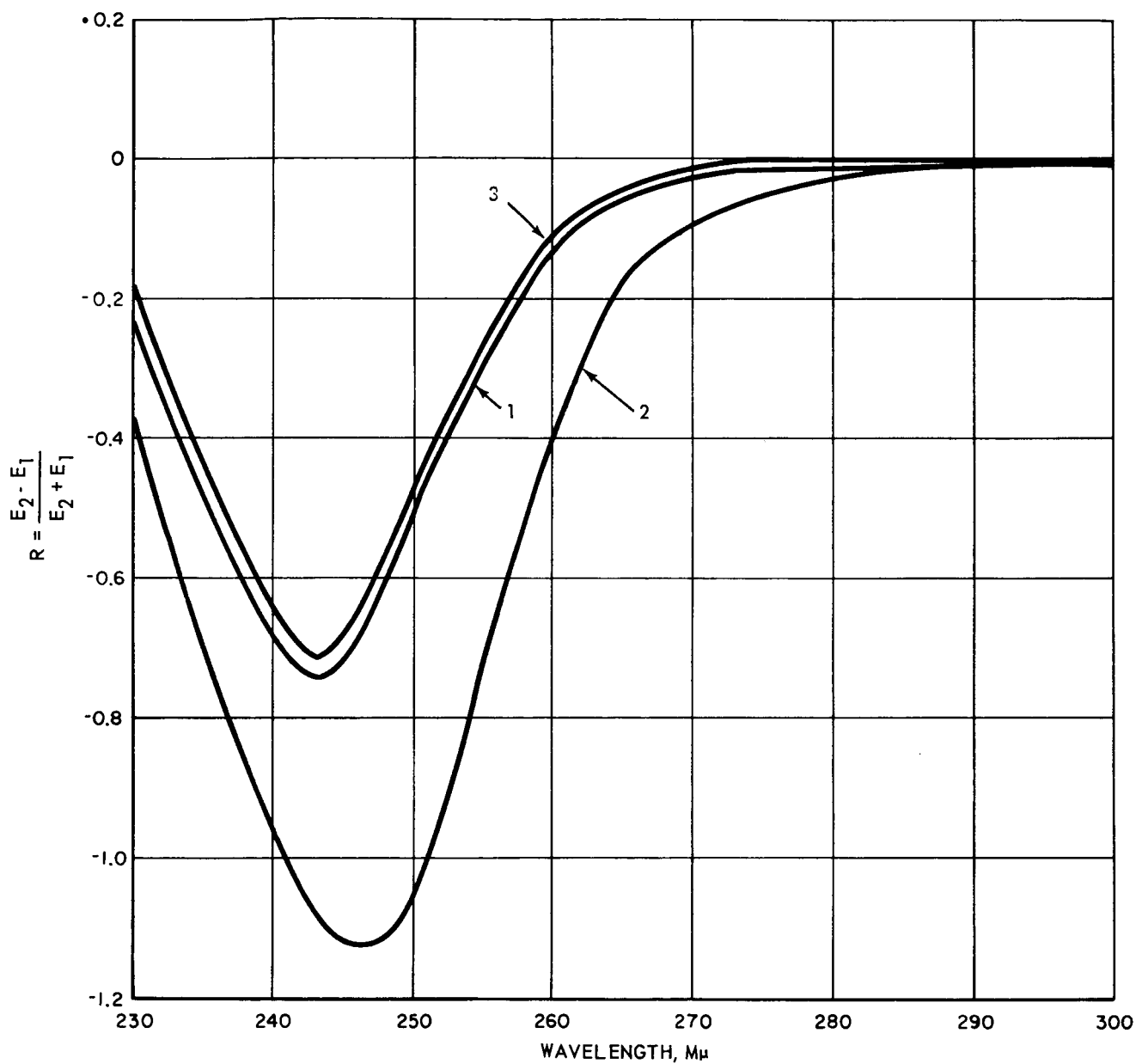


Figure 1. Rotatory Dispersion Spectra of Acidic and Basic Extracts from Soil:  
(1) Initial Acid Extract at  $1 \times 10^{-1}$  gms/ml, (2) Humic Acid Extract  
at  $1 \times 10^{-2}$  gms/ml, and (3) Fulvic Acid at  $2 \times 10^{-2}$  gms/ml.

obtain this dispersion curve, the amount of soil required was twice that required for the humic acid curve. Based upon these observations, it appears that the optically active components within the soils are concentrated within the humic acid fractions.

It has been reported by Marshak and Vogel (3) that the DNA can be hydrolyzed to its purine and pyrimidine bases by treatment with concentrated perchloric acid. The purine and pyrimidine bases are not optically active. Therefore, it was of interest to treat the humic acid fractions in this way to determine what effect the concentrated perchloric acid would have on the dispersion curve of the humic acid fraction. It was expected that any DNA present in the humic acid would be rendered optically inactive. The results of these series of experiments are shown in Figure 2. Curve 1 represents the rotatory dispersion measurements on the soluble humic acid fraction. Curve 2 represents the rotatory dispersion measurements on the perchloric acid treatment of the humic acid fraction. A comparison of curve 1 with curve 2 indicated that some of the optical active components in the humic acid fraction have become optically inactive. It may be noted that the R maximum for the soluble humic acid dispersion curve is approximately 0.78; whereas, the dispersion curve for the perchloric acid treatment of the humic acid has a R maximum of about 0.59. In addition, the amount of the humic acid fraction necessary to produce curve 2 is about 2.5 times the amount of humic acid necessary to produce curve 1.

It was pointed out earlier in this report that the problems of elliptically polarized light and the unequal transmission of the glan

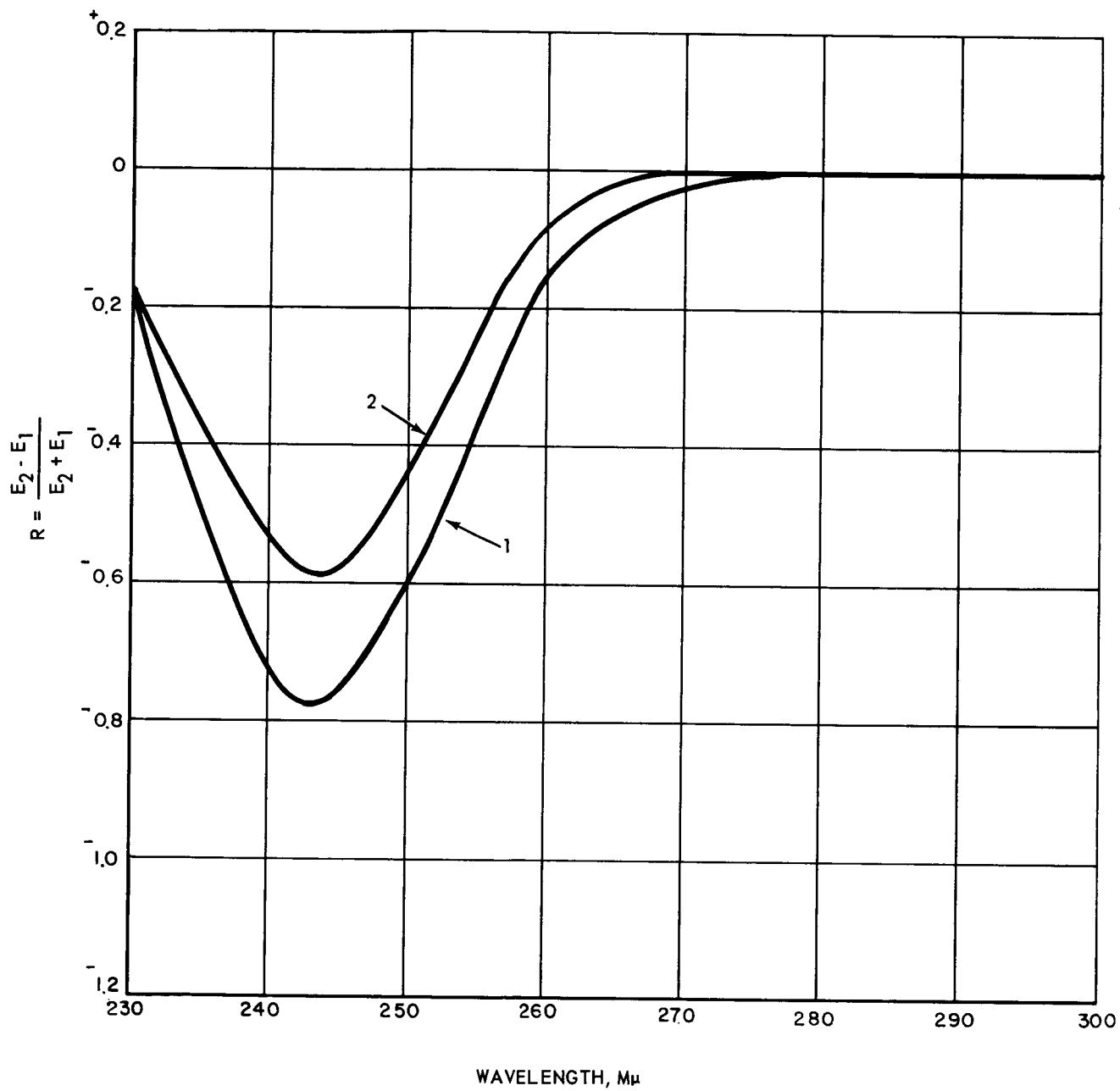


Figure 2. Rotatory Dispersion Spectra of the Humic Acid Fractions of Soil: (1) Basic Treatment at  $4 \times 10^{-4}$  gms/ml and (2) Perchloric Acid Treatment at  $1.0 \times 10^{-3}$  gms/ml.

prism which rendered the Rouy-type polarimeter a qualitative instrument should not effect the measurement of optical activity by using the new electronic ratio, the difference to a constant. To measure this new ratio, the Cary Model 15 spectrophotometer is being used for this purpose. Figure 3 represents the polarizer, sample holder and analyzer arrangement for insertion into the sample chamber of the Cary Model 15 spectrophotometer. Figure 4 represents the polarizer and sample holder arrangement for insertion in the reference chamber of this spectrophotometer. From an examination of the schematics, it is apparent that there are no reflecting surfaces present. Therefore, elliptically polarized light which is generated by the reflection of plane polarized light off of a surface would not be expected.

A mathematical analysis of this system in which account is taken of the unequal transmission of the glan prism indicates this to be no problem either. However, consideration of the unequal transmission of glass prisms in the theoretical discussion of the Rouy-type polarimeter renders its measured ratio, the difference to the sum, a complex function of the angular rotation. (For a complete theoretical discussion of the Rouy-type polarimeter see the proposal submitted by Melpar to NASA on the detection of extraterrestrial life, Method II, Analysis of Optical Rotation Dispersion Profiles, June, 1961.)

The mathematical analysis is as follows:

The equation (1) as shown in Section 2.0 of this report can be readily derived from equation (2). For this derivation see the first quarterly report to NASA under contract NASw-557.

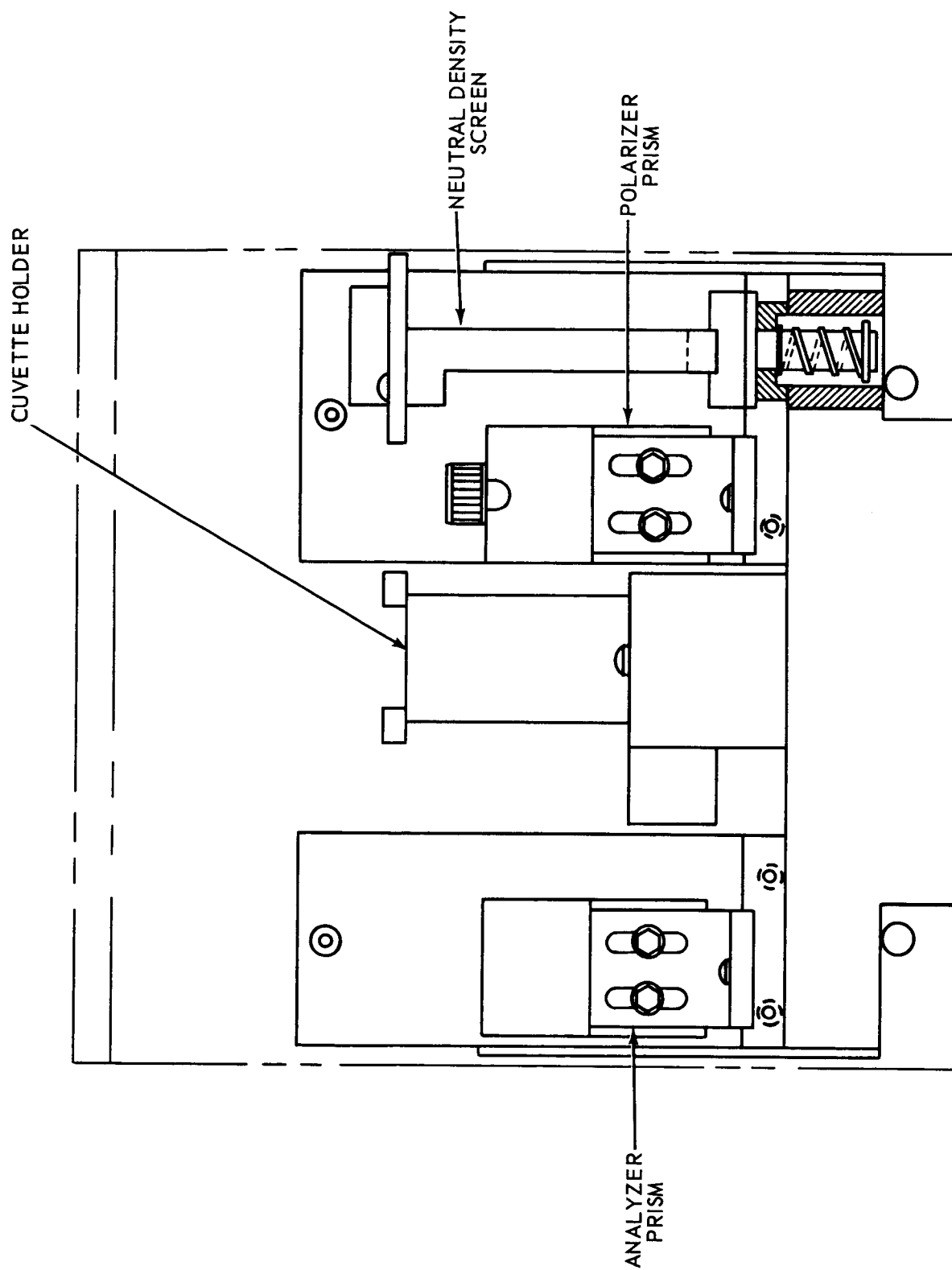


Figure 3. Sample Unit

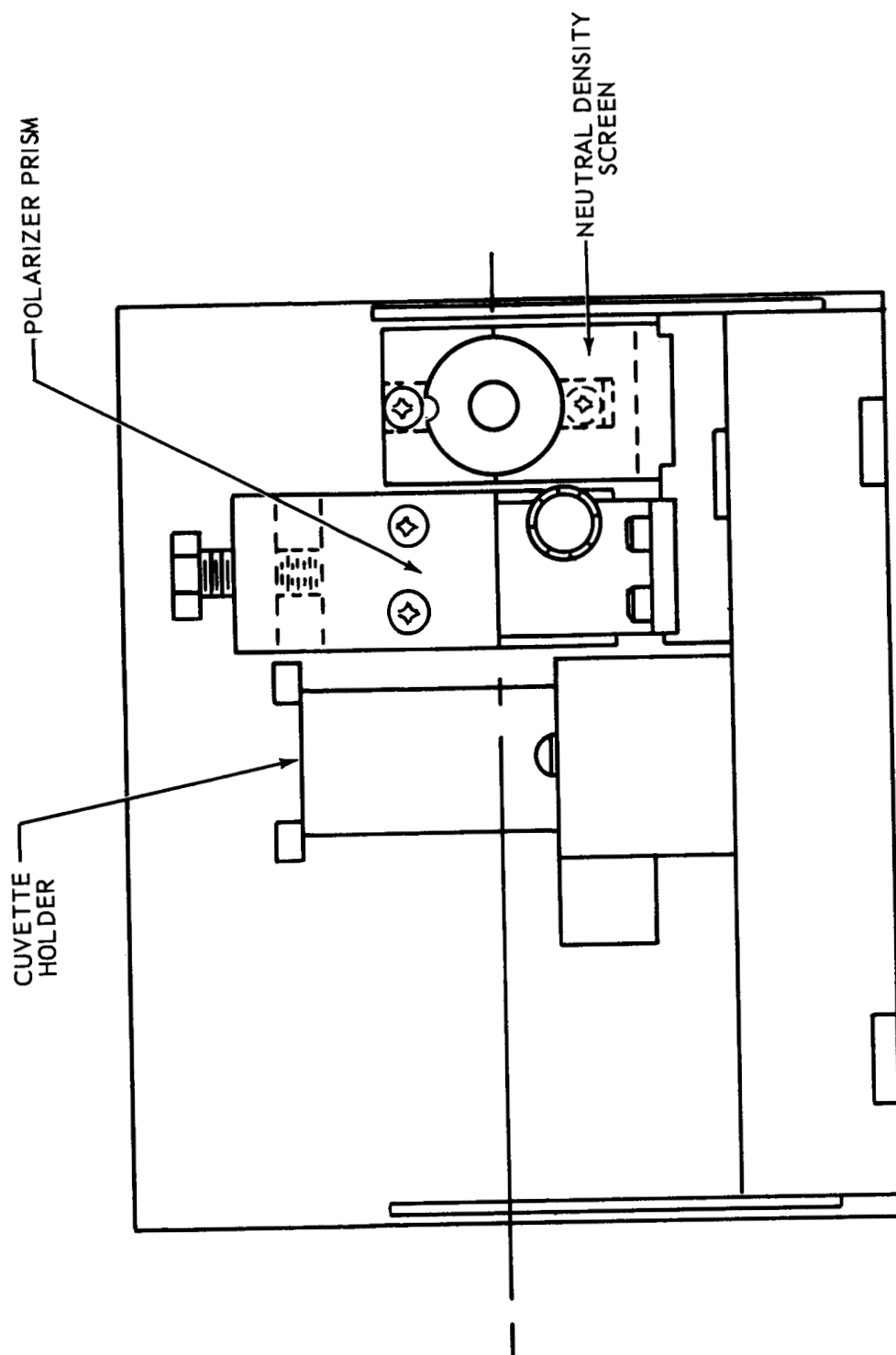


Figure 4. Reference Unit



$$R = \frac{E_1}{E} - \frac{E_2}{E} = \frac{2 \exp(-K_r lc/2) \exp(-K_l lc/2)}{\exp(-K_r lc) + \exp(-K_l lc)} \left[ \frac{\sin 2\theta \sin 2\alpha}{\cos^2 \theta} \right] \quad (2)$$

where

$R$  = ratio of the difference to a constant.

$E_1$  = the light energy which passes through the analyzer in the presence of an optically active sample which exhibits circular dichroism, absorption, and scattering. The optic axis of the analyzer is set at an angle of  $+\theta$  with the optic axis of the polarizer.

$E_2$  = the same conditions as for  $E_1$  only that the optic axis of the analyser is set at an angle of  $-\theta$  with the optic axis of the polarizer.

$E$  = the light energy as seen by a photomultiplier in the presence of only a polarizing prism and the optically active sample.

$K_r, K_l$  = absorption coefficients associated with the absorption or right and left circularly polarized light due to the interaction of an asymmetric environment with a chromophore.

$l$  = path length.

$c$  = concentration of optically active solution.

It should be noted that equation (2) was derived on the basis that the transmission of both glan polarizing prisms were equal.

Let us now consider the derivation of equation (2) in terms of unequal transmission of the glan polarizing prisms. In the determination of the difference to a constant, the ratios  $\frac{E_1}{E}$  and  $\frac{E_2}{E}$  are measured separately. The relationships for the ratios  $\frac{E_1}{E}$  and  $\frac{E_2}{E}$  in the presence of a

non-optically active sample such as water may be written as equation (3) and equation (4).

$$\frac{E_1}{E} = \frac{A^2 \cos^2 \theta}{B^2} \quad (3)$$

$$\frac{E_2}{E} = \frac{A^2 \cos^2 (-\theta)}{B^2} \quad (4)$$

where

$A^2$  = intensity of the light beam emitted from the polarizing prism in the channel containing the analyzer prism.

$B^2$  = intensity of the light beam emitted from the polarizing prism in the channel containing only the polarizing prism.

If an optically active sample which exhibits circular dichroism, absorption, and scattering is placed in both channels, equation (3) and equation (4) may be modified to equation (5) and equation (6), respectively.

$$\frac{E'_1}{E'} = \frac{\frac{A^2}{4} \exp(-k_z l c) \left\{ \left[ \exp(-k_R l c / 2) + \exp(-k_L l c / 2) \right]^2 \cos^2(\theta - \alpha) + \left[ \exp(-k_R l c / 2) - \exp(-k_L l c / 2) \right]^2 \sin^2(\theta - \alpha) \right\} + \Sigma}{\frac{B^2}{2} \exp(-k_z l c) \left[ \exp(-k_R l c) + \exp(-k_L l c) \right]} \quad (5)$$

$$\frac{E'_2}{E'} = \frac{\frac{A^2}{4} \exp(-k_z l c) \left\{ \left[ \exp(-k_R l c / 2) + \exp(-k_L l c / 2) \right]^2 \cos^2(\theta + \alpha) + \left[ \exp(-k_R l c / 2) - \exp(-k_L l c / 2) \right]^2 \sin^2(\theta + \alpha) \right\} + \Sigma}{\frac{B^2}{2} \exp(-k_z l c) \left[ \exp(-k_R l c) + \exp(-k_L l c) \right]} \quad (6)$$

where:

$\Sigma$  = amount of polarized light passing through the analyzer because scattering results in depolarization of plane polarized light.

$K_{\Sigma}$  = scattering coefficient

$\alpha$  = angular rotation produced by optically active solution

Dividing equation (3) into equation (5), equation (4) into equation (6) and cancellation of common terms in both numerator and denominator results in equation (7) and equation (8), respectively.

$$\frac{E'_1/E_1}{E'_1/E_1} = \frac{\frac{1}{2} \{ [\exp(-k_R l_c/2) + \exp(-k_L l_c/2)]^2 \cos^2(\theta - \alpha) + [\exp(-k_R l_c/2) - \exp(-k_L l_c/2)]^2 \sin^2(\theta - \alpha) \}}{[\exp(-k_R l_c) + \exp(-k_L l_c)] \cos^2 \theta} + \frac{2\Sigma}{A^2 \exp(-k_L l_c) [\exp(-k_R l_c) + \exp(-k_L l_c)] \cos^2 \theta} \quad (7)$$

$$\frac{E'_2/E_2}{E'_2/E_2} = \frac{\frac{1}{2} \{ [\exp(-k_R l_c/2) + \exp(-k_L l_c/2)]^2 \cos^2(\theta + \alpha) + [\exp(-k_R l_c/2) - \exp(-k_L l_c/2)]^2 \sin^2(\theta + \alpha) \}}{[\exp(-k_R l_c) + \exp(-k_L l_c)] \cos^2 \theta} + \frac{2\Sigma}{A^2 \exp(-k_L l_c) [\exp(-k_R l_c) + \exp(-k_L l_c)] \cos^2 \theta} \quad (8)$$

Subtracting equation (8) from equation (7), taking advantage of the trigonometric identities, and simplification

$$\cos^2 X + \sin^2 X = 1$$

$$\cos^2 X - \sin^2 X = \cos 2X$$

$$\cos (X \pm Y) = \cos X \cos Y \mp \sin X \sin Y$$

leads to equation (9)

$$\frac{E'_1/E_1}{E'_2/E_2} - \frac{E'_2/E_2}{E'_1/E_1} = \frac{2 \exp(-k_R l_c/2) \exp(-k_L l_c/2)}{\exp(-k_R l_c) + \exp(-k_L l_c)} \left[ \frac{\sin 2\theta \sin 2\alpha}{\cos^2 \theta} \right] \quad (9)$$

It should be noted that equation (9) is identical with equation (2); therefore, consideration of the unequal transmission of the glan polarizing prisms indicates no problems in measuring angular rotation by means of the electronic ratio of, the difference to a constant.

## 5.0 CONCLUSIONS

The optical rotatory dispersion measurements performed on an initial acid extract, the humic acid extract, and the fulvic acid extract of soil indicated that the optically active components within the soil are concentrated primarily in the humic acid or base soluble fraction. Treatment of this fraction with concentrated perchloric acid showed a decrease in optical activity. This result suggests the possibility of acid hydrolysis of some of the optically active components.

Work will be continued on the analysis of the humic acid fraction by chromatographic means to determine what components in this fraction

are optically active, and also to determine what optically active components are hydrolyzed by the concentrated perchloric acid. Should it be shown that the DNA and its congeners are the primary reactants of the acid hydrolysis reaction, a simple and rapid method for detecting the amount of the substances in soil may be evolved.

It has been pointed out that the measurements of optical activity with the Rouy-type polarimeter are quantitative. This is due to the fact that the glan polarizing prisms transmit unequal amounts of light. Also, the reflecting of plane polarized light off a surface results in the formation of elliptically polarized light. This light is capable of passing through an analyzer prism unattenuated. Mathematical considerations of these problems in the measurement of optical rotation by means of the new electronic ratio, the difference to a constant, have indicated that they may be neglected.

Ref. (3) Marshak, A., and Vogel, H.J., J. Biol. Chem., 189, 597 (1951)